applicants' invention, Claim 20 has been amended to recite that "the presence of at least about 20 U/L erythrocyte adenylate kinase activity in said sample [is] indicative of erythrocyte hemolysis in said subject". Applicants submit that "at least about 20 U/L" is supported by the application as filed.

Specifically, Table 1 provides a summary of the levels of hemoglobin in multiple serum samples taken from patients suspected of having hemolysis. In Table 1, samples 1-15 were hemolyzed, while samples 16-20 were not. The hemoglobin level for samples 1-15 ranged from 1-6 g/L, while the hemoglobin level for samples 16-20 was zero. Therefore, hemolysis requires the presence of at least 1 g/L hemoglobin. The corresponding level of total adenylate kinase in each sample is also disclosed in Table 1. However, since total adenylate kinase includes adenylate kinase from erythrocytes as well as other sources (e.g. muscle) (and, thus, may not represent an accurate level of hemolysis in the sample), Figure 3 is helpful in correlating the levels of hemoglobin to erythrocyte adenylate kinase activity. As shown in Figure 3, about 0.1 g/dL hemoglobin (i.e. 1 g/L hemoglobin shown in Table 1 to represent the minimal level of hemoglobin in which hemolysis is present) corresponds to about 20 U/L erythrocyte adenylate kinase activity. Therefore, based upon this conversion, hemolysis in the serum samples from patients corresponds to at least about 20 U/L erythrocyte adenylate kinase activity.

In addition, applicants describe at page 11, lines 22-28 that the level of erythrocyte adenylate kinase activity "was found to be linear between the ranges of 0-120 U/L and was proportional to hemoglobin concentrations between 0 and 5 g/L as shown in Figure 3", and "[a]bout 1 U/L erythrocyte adenylate kinase activity is equal to about 0.005 g/DL hemoglobin concentration." Here again, using this conversion ratio set forth at page 11, lines 26-28 of the specification, 1 U/L hemoglobin concentration (as set forth in Table 1) correlates with about 20 U/L erythrocyte adenylate kinase activity

(i.e. (1 U/L hem.) x (1 / .05g/L hemoglobin) x (1 U/L eryth. AK activity) = 20 U/L eryth. AK activity). Therefore, based upon this conversion, hemolysis in the serum samples from patients corresponds to at least about 20 U/L erythrocyte adenylate kinase activity.

For these reasons, applicants submit that the claim language "at least about 20 U/L" is supported by the application as originally filed. Accordingly, reconsideration and withdrawal of this rejection is respectfully requested.

35 U.S.C. §103 Rejections

Claim 20 was also rejected under 35 U.S.C. §103 as unpatentable over Olsson. Applicants respectfully traverse this rejection, and maintain that Claim 20 is patentable over Olsson.

Olsson detected elevated levels of hemoglobin and adenylate kinase in various samples subjected to different periods of storage. Specifically, Olsson found some correlation between the amount of accumulated hemoglobin and adenylate kinase in samples of whole blood and red blood cell concentrates following storage (see Figure 6 of Olsson). Olsson also measured an increase in the accumulation of extracellular adenylate kinase during storage of platelets (see Figure 7 of Olsson). It is worth noting that although Olsson found some correlation between hemoglobin and adenylate kinase, the ratio of hemoglobin to extracellular adenylate kinase between red blood cell concentrates depicted in Figures 6A and 6B was significantly different (see pages 442 and 445 of Olsson).

In addition, although Olsson suggested that measuring the level of adenylate kinase may be useful for studying the lytic processes of platelets, and possibly the testing

of blood cell packs prior to transfusion, there is absolutely no teaching or suggestion in Olsson to use erythrocyte adenylate kinase activity for diagnosing erythrocyte hemolysis that takes place *in vivo*. In this regard, it is worth noting that Olsson provides no experimental evidence to support that erythrocyte adenylate kinase activity actually correlates with hemolysis *in vivo*. In addition, even assuming there is a correlation *in vivo*, Olsson provide no teaching or suggestion that the erythrocyte adenylate kinase released in the patient's blood is stable and is not taken up by any tissues or cells or is in any way extruded from the patient's body that would make such a correlation meaningful and detectable.

For these reasons, applicants submit that the claimed invention is patentable over Olsson. Accordingly, reconsideration and withdrawal of this rejection, and allowance of Claim 20, are respectfully requested.

No fee, other than the $55.00 one month extension of time fee, is deemed necessary in connection with the filing of this Amendment. If any fee is required, authorization is hereby given to charge the amount of any such fee to Deposit Account No. 01-1785.

Respectfully Submitted,

AMSTER, ROTHSTEIN & EBENSTEIN
Attorneys for Applicants
90 Park Avenue
New York, New York 10016
(212) 697-5995

Dated: March 6, 2000
New York, New York

By _____
Craig J. Arnold, Reg. No. 34,287